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Temperature, Salinity, and Aerial Exposure Tolerance of the Invasive Mussel, *Perna viridis*, in Estuarine Habitats: Implications for Spread and Competition with Native Oysters, *Crassostrea virginica*

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Abstract Temperature, salinity, and aerial exposure tolerances of the invasive green mussel, *Perna viridis*, were investigated under environmentally realistic conditions to address potential competition with native oysters, *Crassostrea virginica*. This study demonstrated that green mussels exhibit a temperature tolerance range similar to that of its native range (13–30 °C), thereby limiting northern spread of south Florida populations. Salinity tolerances as low as 6 were observed during gradual decreases; however, acute changes resulted in low survival at salinities of 15 and below. At low salinities, even when survival was high, reductions in normal behavior (byssal production and valve closure) were observed. Green mussel survival was significantly inhibited during aerial exposure across all temperatures, and mortality synergistically increased (60–80 %) with increasing temperatures and exposure time. In contrast, oysters demonstrated ≥97 % survival over the exposure period at similar internal temperatures observed in green mussels. Internal temperatures for both species frequently exceeded 33 °C, reaching temperatures as high as 41 °C, while air temperatures averaged only 26.4±1.6 °C. Our results suggest that while Florida green mussel populations exhibit relatively broad temperature and salinity ranges, their spread is limited by the available subtidal habitat, potentially sparing intertidal oyster reefs from habitat and nutrient competition.

Keywords Desiccation · Osmotic equilibrium · Heat shock · Non-indigenous

Introduction

The green mussel *Perna viridis* is a recent marine invader to southwest Florida. It was first observed in Tampa Bay in the Gulf of Mexico in 1999 (Benson et al. 2001; Ingrao et al. 2001) and has since spread south along the Gulf coast to Marco Island and along the Atlantic coast from Palm Beach Gardens, FL, north to Charleston, SC (Baker et al. 2007; Rajagopal et al. 2006). Since their first appearance in the Caribbean, dense populations have spread throughout the coastal waters of Trinidad and Tobago, Venezuela, Jamaica, and Cuba (Agard et al. 1992; Rylander et al. 1996; Buddo et al. 2003; Fernández-Garcés and Rolán 2005). Molluscan invaders, such as *P. viridis*, can have large impacts on native bivalve populations, as exemplified by the invasion of the zebra mussel *Dreissena polymorpha* in the Great Lakes, which contributed to a reduction in native species, as well as economic loss due to excessive infrastructure biofouling (Baker and Hornbach 2000; Connelly et al. 2007; Schloesser and Nalepa 1994).

There is concern that *P. viridis* will decimate critical oyster reef habitat. Green mussels occupy hard substrate in Tampa Bay, particularly those which are manmade (Baker et al. 2012), including coastal buoys and hard substrates on the

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outer fringes of the bay (Volety et al., unpublished results). However, green mussels have also been observed on oyster reefs within the bay (Baker et al. 2006; Volety et al., unpublished results) and have contributed to the apparent displacement of the native oyster, *Crassostrea virginica* (Baker et al. 2012). Green mussels and oysters can provide ecosystem services, such as benthic-pelagic coupling, but green mussels lack the ability to create the permanent and complex three-dimensional habitat that oyster reefs provide to many economically and ecologically important estuarine invertebrates and fishes (Abeels et al. 2012; Tolley and Volety 2005; Wells 1961). Furthermore, the loss of oyster reefs has become a worldwide problem (NOAA 2005; Beck et al. 2011).

Organisms that inhabit estuaries, especially those that are sessile and intertidal, must be tolerant of extreme and sometimes rapid variations in environmental conditions. Estuaries in southwest Florida experience dramatic salinity changes between dry and wet seasons; waters can be hypersaline (28–38) in the winter months, quickly transitioning to nearly fresh conditions (0–10) during the summer rainy season (Barnes et al. 2007; Volety et al. 2009). Water temperatures are less volatile but still vary seasonally; winter water temperatures average 13–18 °C and summer water temperatures average 30–32 °C, with highs reaching 35 °C (Badylak et al. 2007; Barnes et al. 2007). Organisms on intertidal oyster reef systems must cope not only with temperature extremes but with aerial exposure as well. During aerial exposure in the summer months, the daily variation in body temperature of bivalves may increase by over 20 °C, exceeding ambient air temperature (Hofmann and Somero 1995; Stillman and Somero 2000).

The distribution limits and competitive advantage of a species will be set by its ability to tolerate the environmental extremes to which it is exposed (Gosling 2008). The temperature and salinity tolerances of *C. virginica* are well studied, particularly for temperate populations (Shumway 1996). Temperature and salinity tolerances of *P. viridis* are also known from their native range in the Indo-Pacific (Sivalingam 1977; Vakily 1989); however, environmental tolerances may vary by geographic location and between native and invasive populations (Baker et al. 2012; Shumway 1996). In addition, little is known concerning the comparative tolerances of *C. virginica* and *P. viridis* to aerial exposure. Our overall goals, therefore, were to examine the potential for the green mussel to thrive in local environmental conditions and to evaluate the potential for spread in southwest Florida estuaries and its ability to successfully compete with the native oyster. Our specific objective was to assess (1) the temperature tolerance of *P. viridis* from southwest Florida, (2) the survival of *P. viridis* following gradual and acute salinity declines, and (3) the comparative aerial exposure tolerance of both *P. viridis* and *C. virginica*.

Methods

Temperature Tolerance

We examined the temperature tolerance of green mussels, *P. viridis*, from southwest Florida. Mussels were hand collected from pilings at a depth of 3–4 m using SCUBA at Gandy Bridge in Tampa Bay, FL (Fig. 1). Green mussels were collected two times; those collected in July 2002 had previously survived a range of high summer temperatures and were termed “summer-acclimatized,” and those collected in March 2003 had previously survived a range of low winter temperatures and were termed “winter-acclimatized.” Specimens were transported to Mote Marine Laboratory in Sarasota, FL, cleaned of epiphytic growth, and held for 72 h unfed in filtered seawater at harvest temperature and salinity.

Experiments were conducted in ten recirculating 115-L tanks, two tanks for each of five temperature treatments. Water temperatures in each tank were maintained with TECO chiller-heaters (model RA 200, Ravenna, Italy) connected to Quiet One Pro Aquarium Pumps (LifeGard Aquatics, Cerritos, CA), with the addition of aquarium heaters, as needed. Mussels were placed into glass jars (470 mL) covered with a 1-cm mesh held in place with a rubber band. Eighteen jars per treatment ($N=3$ per jar) were randomly submerged in each of five treatment tanks (one tank per treatment). Seawater was delivered to the bottom of each glass jar via tubing attached to a PVC manifold and Quiet One pump. Flow rates were set to 250–300 mL min^{-1}. Mussels were not fed during experimentation. Water quality parameters (temperature, salinity, dissolved oxygen, pH, ammonia, nitrite) were monitored daily.

Summer-acclimatized *P. viridis* were collected from the field at a salinity of 23 and 30 °C. Salinity was maintained at 23 for the duration of the experiment using bay water. Temperature in the treatment tanks was adjusted by 3–4 °C every 24 h until the desired test temperatures were reached: 20, 25, 30, 35, and 40 °C. Every 24 h, the jars were removed from the tanks, without draining, and moved to adjacent tanks that had been prepared at the appropriate temperatures and salinities. Observations of *P. viridis* mortality (failure to remain closed when prodded with a pipette) were conducted at 1 h (tanks at 40 °C) or at 12-h intervals (all other tanks). Higher incidence of mortality in the 40 °C treatment warranted more frequent monitoring. Once tank temperatures reached the final target temperatures, experiments were monitored for an additional 10 days.

Winter-acclimatized *P. viridis* were collected from the field at a salinity of 20 and 22 °C. Salinity was maintained at 20 for the duration of the experiment using bay water. Temperature in the treatment tanks was adjusted by 3–4 °C every 24 h until the desired test temperatures were reached: 10, 13, 16, 22, and 30 °C. Once tank temperatures reached the final target

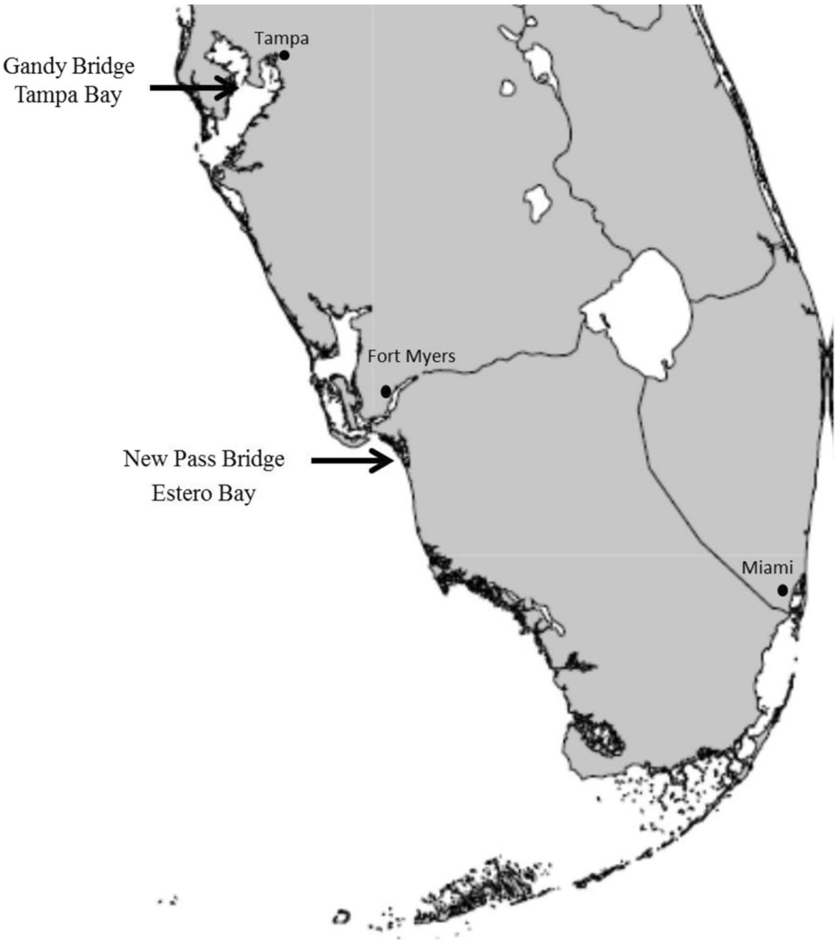

Fig. 1 Map showing the collection site used for collection of organisms

temperatures, experiments were monitored for an additional 12 days. Jars were transferred between tanks, and mortality was recorded, as described above.

Performance of *P. viridis* in the temperature tolerance experiments was examined graphically as cumulative mortality over time. In addition, mean survival time (MST) was calculated for each temperature treatment using Kaplan-Meier survival estimates (StatsDirect v. 2.7.9).

Tolerance of Acute Salinity Decline

We examined the survival of *P. viridis* following acute decline in salinity. Mussels were collected from New Pass Bridge in Estero Bay, FL, in August 2010, immediately cleaned of epiphytic growth, and kept in holding tanks with recirculating filtered seawater at a salinity of 30 (the salinity at which they were collected) for 1 week prior to experimentation. Due to the extended exposure time of salinity experiments, mussels were fed Shellfish Diet® (Reed Mariculture Inc., Campbell, CA) at a rate of ~1 mL per individual per day. Desired salinities were achieved using filtered seawater and adjusting with either Instant Ocean™ (Aquarium Systems Inc., Cincinnati, OH) or deionized water.

Experiments were conducted in 40-L tanks, with three replicate tanks for each of seven salinity treatments: 5, 10, 15, 20, 25, 30, and 35. Specimens were removed from holding tanks (salinity of 30) and placed directly into the salinity treatments, ten mussels per tank. Tanks were maintained at the test salinity for 56 days, with water changes completed every other day. Survival was monitored daily (as described above) with the immediate removal of dead mussels to avoid degradation of water quality.

Survival of *P. viridis* to acute changes in salinity was examined using repeated measures ANOVA after applying an arcsin-square root transformation, and a multiple comparisons test (Tukey's honest significant difference (HSD)) was used to identify differences in mean survival between specific salinity treatments (SPSS 19). In addition, MST was calculated for each salinity treatment using Kaplan-Meier survival estimates (SPSS 19).

Tolerance of Gradual Salinity Decline

We determined the survival of *P. viridis* during and following chronic, gradual declines in salinity. Mussels were collected from New Pass Bridge in Estero Bay, FL (Fig. 1), in February

2011 and maintained as for the acute salinity exposure. All treatments (three replicates per treatment; 12 individuals per replicate) started at an initial salinity of 30. Salinity was decreased by 3 every other day until the desired treatment salinity was reached, with the lowest salinity treatment (3) requiring 19 days. For each salinity decrease, a full water change was completed on all tanks and temperature was kept constant at 22±1 °C throughout the exposure. Once all test salinities (30, 27, 24, 21, 18, 15, 12, 9, 6, 3) were reached, tanks were maintained for an additional 28 days. Full water changes were conducted every other day in order to maintain experimental conditions and water quality. Survival was monitored daily (as described above) with the immediate removal of dead individuals. In addition, byssal thread production and valve closure/opening behavior were noted. Those individuals no longer attached to the walls of the tank or forming clumps with other mussels were considered to have decreased byssal thread production. Specimens in which valves were open and gills were clearly visible and actively filtering were considered to be open (normal behavior), while those with no observable opening were considered to have closed valves.

Survival of *P. viridis* to gradual changes in salinity was examined using repeated measures ANOVA after applying an arcsin-square root transformation, and a multiple comparisons test (Tukey's HSD) was used to identify differences in mean survival between specific salinity treatments (SPSS 19). In addition, MST was calculated using Kaplan-Meier survival estimates (SPSS 19) for each salinity treatment, with the day all test salinities were reached (day 19) as time zero to avoid artificially inflating survival time (≥10 days) during exposure to salinities within their tolerance range.

Aerial Exposure Tolerance

We examined the internal temperature and survival of both *P. viridis* and *C. virginica* during aerial exposure. *P. viridis* were collected in September 2011 from a subtidal fender system at New Pass Bridge in Estero Bay, FL (Fig. 1), and *C. virginica* from an intertidal reef just inside New Pass Bridge. In Estero Bay, oysters are primarily found on intertidal reefs, while green mussels are only found on subtidal artificial substrate. All animals were held in subtidal conditions in the laboratory for 2 weeks prior to experimentation, and new individuals were chosen each day of testing. Internal body temperature of three individuals per species was monitored on nine different days. A hole (1.5 mm) was drilled into the posterior portion of one valve of each specimen, an OMEGA HH12B digital thermometer probe (OMEGA Engineering, Inc., Stamford, CT) was inserted through the hole, and the hole was sealed with cyanoacrylate adhesive (Super Glue®). Individuals were arranged on top of dead oyster shell and placed in full sun to simulate an intertidal oyster reef. Surface temperature was measured using an aquarium thermometer placed on top of the exposed oyster shell, and air temperature was obtained from a local meteorological station (http://www.wunderground.com/weatherstation). Both internal and surface temperatures were recorded every 20 min, beginning at 9:00 a.m. each day and concluding at 3:30 p.m. Survival of oysters and green mussels was noted during exposures and expressed as cumulative survival. To account for the possibility of drilling contributing to *P. viridis* mortality during the aerial exposures, further exposures were conducted with undrilled specimens. On three separate days, 12, 6, and 12 green mussels, without shell holes or thermometer probes, were monitored under similar test conditions as above and both surface and air temperature recorded. A Student's *t* test was used to identify differences in mean survival between species and multiple regression analysis used to determine the effect of time and temperature on survival (SPSS 19).

Results

Temperature Tolerance

Collection season (July or March) had an effect on *P. viridis* response to temperature challenges (Fig. 2a, b). At the common temperature of 30 °C in both experiments, summer-acclimatized mussels had 100 % survival over the 10-day experiment (Fig. 2a), while winter-acclimatized mussels had only 46 % survival over the 12-day exposure (MST=8.4 days) (Fig. 2b). Examining the results of the summer- and winter-acclimatized experiments together, *P. viridis* appear to have lower and upper lethal limits of ≤10 and >35 °C, respectively. All summer-acclimatized mussels held at 40 °C died within 24 h (MST=1 day), while those held at 35 °C had high survival through the ninth day of exposure but suffered 100 % mortality by day 10 (MST=9.1 days). Winter-acclimatized mussels subjected to 10 °C for 12 days experienced mortalities of 93 % (MST=8.8 days). Mortalities were low at temperatures between 10 and 35 °C; summer-acclimatized mussels had 100 % survival at 20, 25, and 30 °C, while winter-acclimatized mussels had >80 % survival at 13, 16, and 22 °C (MST=11.9, >12.0, and 10.3 days, respectively) over the exposure period.

Tolerance of Acute Salinity Decline

Acute salinity treatment had a significant effect on mortality of *P. viridis* ($p \leq 0.001$) (Fig. 3) with poor survival when exposed to acute salinity changes of 15 or more. Mussels transferred from 30 to salinities of 5 and 10 suffered 100 % mortality within 13 days (MST=9.1 and 8.8 days, respectively). Survival in these two treatments was significantly lower than

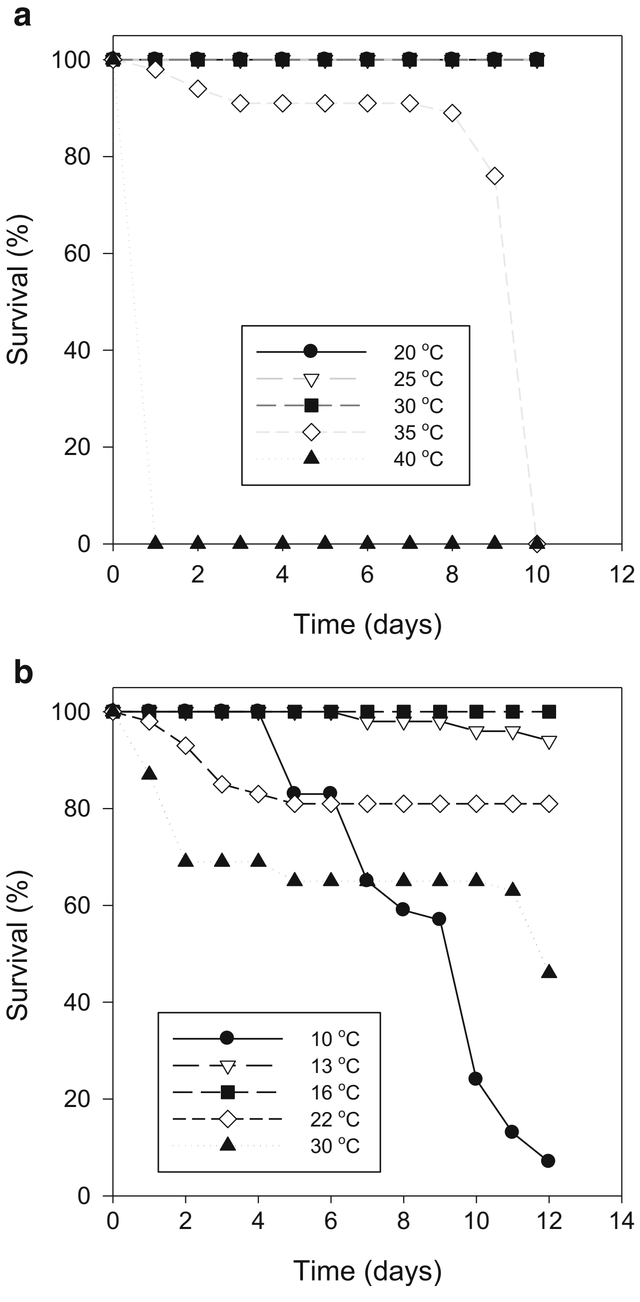

Fig. 2 **a** Cumulative survival of summer-acclimatized green mussels over time in response to a change in temperature. **b** Cumulative survival of winter-acclimatized green mussels over time in response to a change in temperature

that in all other treatments (Tukey's HSD, $p \leq 0.001$). Survival in the salinity treatment of 15 was also significantly different from all other treatments (Tukey's HSD, $p \leq 0.001$); 20 % of those transferred to a salinity of 15 remained alive at the end of the 8-week experiment (MST=22.1 days). Mortalities were low when the acute change in salinity was 10 or less; *P. viridis* acclimated to a salinity of 30 had >85 % survival at 20, 25, 30, and 35. However, survival in the salinity treatment of 35 was significantly lower (Tukey's HSD, $p \leq 0.001$) than those in the 20 and 25 salinity treatments, which had 100 % survival over the 56-day exposure.

Tolerance of Gradual Salinity Decline

Gradual changes in salinity had a significant effect on *P. viridis* mortality ($p \leq 0.001$); however, *P. viridis* were relatively tolerant of gradual declines in salinity. Survival at salinity treatments of 9 and greater had survival rates of >97 % throughout the experiment (Fig. 4). Only mussels exposed to salinity treatments of 3 and 6 had significantly lower survival than all other treatments (Tukey's HSD, $p \leq 0.001$); those in the salinity treatments of 3 suffered 100 % mortality within 13 days of reaching the test salinity (MST=8.5 days), while those in the salinity treatments of 6 had only 47 % mortality after 28 days at the test salinity (MST=17.7 days). We also observed changes in behavior at salinities of 9 or less. Mussels exposed to salinity treatments of 3, 6, and 9 exhibited decreased byssal attachment, lying loosely on the bottom of the tanks, and increased valve closure periods, resulting in decreased water clarity after feedings. Mussels in salinity treatments of 12 or greater, however, formed tight clumps and strong attachment to the walls of the aquaria with gills exposed and actively filtering, resulting in high water clarity shortly after the addition of food.

Aerial Exposure Tolerance

P. viridis suffered significantly greater mortalities ($t(34) = -4.084$; $p = 0.004$) than *C. virginica* in response to aerial exposure, despite reaching similar internal temperatures within the same time period (Fig. 5). Exposure time was the most significant predictor of *P. viridis* mortality ($F_{(1,39)} = 145.227$; $p \leq 0.001$; $R^2 = 0.793$). Mussel mortalities began within 2 h of initial exposure, when internal temperatures reached 34.8±3.5 °C. *P. viridis* mortality increased with increasing temperature and exposure time; at the end of a 6-h aerial exposure, cumulative mortality (Fig. 6) was 60 % with an overall average internal temperature of 33.6±1.9 °C. In contrast, only two *C. virginica* died during the aerial exposure experiments (4 % mortality). Internal temperatures of both *P. viridis* and *C. virginica* were similar to each other, slightly higher than surface temperature, and significantly higher than air temperature. Similar results were observed during the survival trials for *P. viridis* with no holes or temperature probes. Mortalities were observed within 1 h of aerial exposure independent of temperature, and mortalities were observed at surface temperatures as low as 33 °C. Mortality increased as temperature increased; after 4.5 h of aerial exposure, 83.3±14.4 % were dead with an average surface temperature of 35.9±3.4 °C (Table 1).

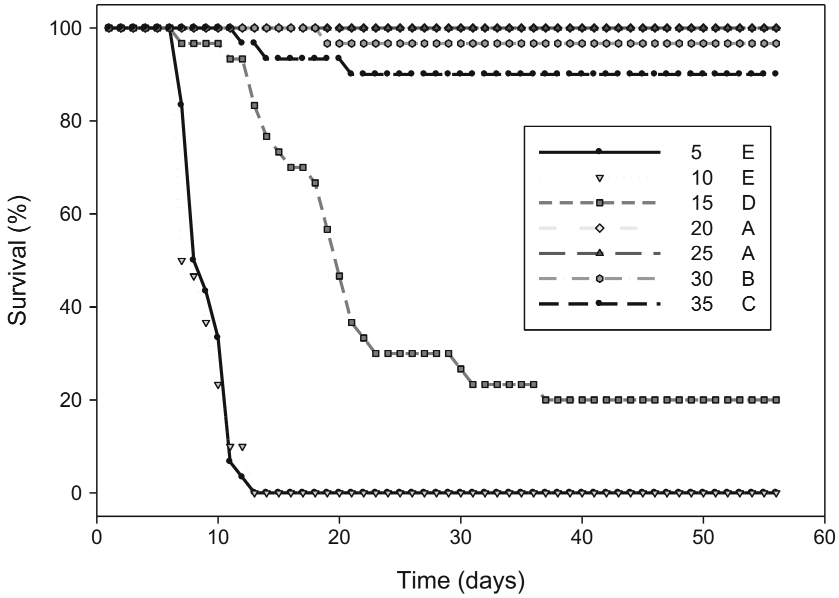

Fig. 3 Survival of green mussel in response to an acute decrease in salinity over time. *Letters* denote significance between treatments

Discussion

We found that *P. viridis* from southwest Florida tolerated a broad range of temperatures and were resistant to gradual salinity changes. However, both acute salinity changes greater than 15 and aerial exposures resulted in significant mortality. Our data will contribute to predicting the success and potential spread of *P. viridis* in estuaries of the southeastern USA, as well as their ability to compete with the native oyster, *C. virginica*, and impact critical oyster reef habitat.

The results of our laboratory studies suggested that the southwest Florida populations we sampled had a temperature tolerance range (13–30 °C) that is comparable to, although not identical to, temperature tolerance ranges published for both native and invasive populations. For *P. viridis* in the native range, Sivalingam (1977) reports 50 % survival at 10 and 35 °C and optimum temperatures of 26–32 °C. However, invasive populations may represent only a fraction of the source population, with a subset of the overall genetic diversity, or may represent multiple introductions, with high genetic diversity (Kolbe et al. 2004; Ruis et al. 2014; Sakai et al. 2001). Therefore, physiological tolerances of introduced populations may differ from those of populations in the native range. *P. viridis* were introduced into Japanese waters in 1967, and winter survival rates indicate that they do not survive extended periods below 12 °C (Ueda et al. 2013). Segnini de Bravo et al. (1998) report low and high lethal temperatures of 6 and 37.5 °C for Venezuelan populations, introduced in 1993. Field studies in Tampa Bay, where the species was first observed in the USA in 1999, demonstrate that subtidal

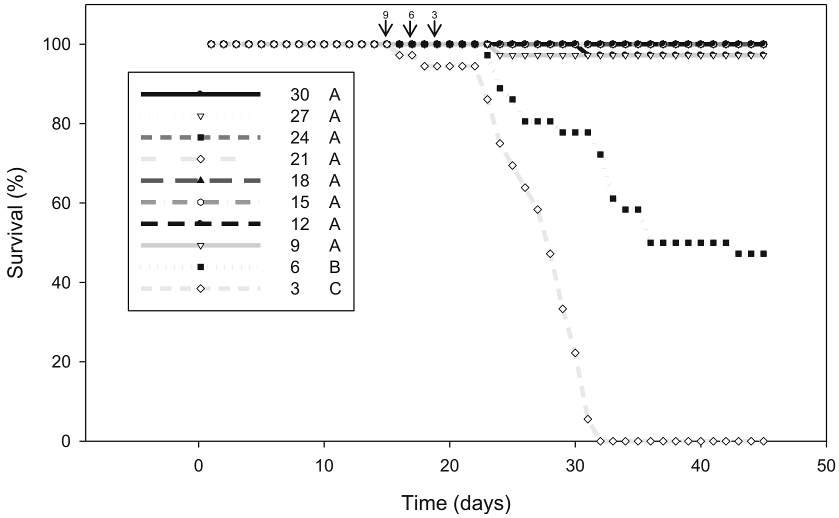

Fig. 4 Survival of green mussels over time in response to a gradual decrease in salinity. *Arrows* mark the time in which salinities of 9, 6, and 3 were reached, and *letters* denote significance among treatments

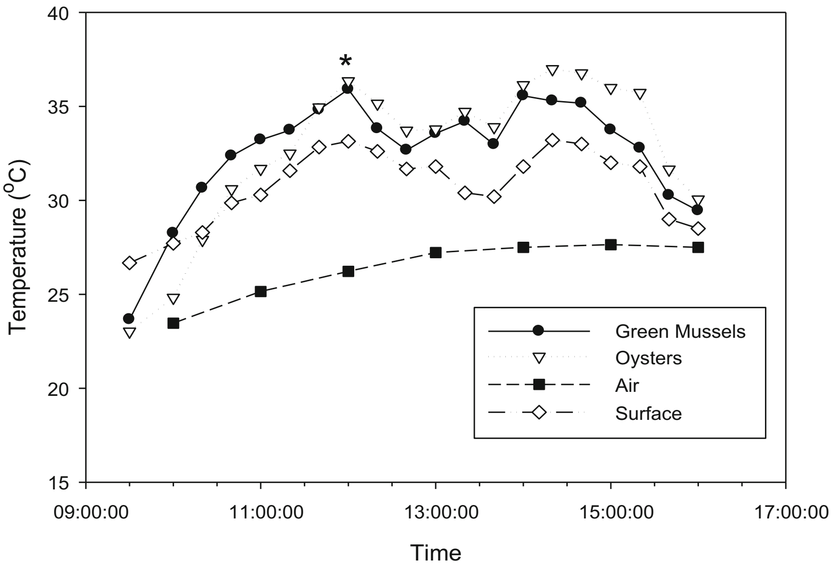

Fig. 5 Graphical representation of the internal temperatures of mussels and oysters compared with surface and air temperatures over time. *Asterisk* marks the time when green mussel mortalities begin

P. viridis are unaffected by winter temperatures as low as 12.9 °C (Baker et al. 2012). To determine the potential for northward range expansion in the southeastern USA, Urian et al. (2011) examined the lower thermal limits of *P. viridis* from northeast Florida and reported a lower temperature threshold between 10 and 14 °C.

The variety of experimental methods employed, and field or laboratory acclimatization/acclimation history, may explain the range of temperature tolerance measured for *P. viridis* in our studies, compared to previously published work. Recent thermal history plays an important role in determining the induction temperatures of heat-shock gene expression and, therefore, temperature tolerance in invertebrates (Buckley et al. 2001; Hamdoun et al. 2003; Osovitz and Hofmann 2005). Our lower and upper temperature tolerances were determined following winter or summer acclimatization in the field, and laboratory tanks were adjusted by 3–4 °C every 24 h until reaching the desired test temperatures. In contrast, Segnini de Bravo et al. (1998), for example, acclimated *P. viridis* at 25 °C for 15 days, followed by temperature increases or decreases of 1 °C per day until the upper or lower lethal limits were reached. Urian et al. (2011) collected *P. viridis* in December, acclimated them at 14 °C for 14 days, and conducted acute chill resistance experiments at 14, 10, and 3 °C. Other published temperature limits, particularly those for lower limits and optimum range, are inferred from field data (Baker et al. 2012; Sivalingam 1977; Ueda et al. 2013). Our combination of field acclimatization and laboratory acclimation may have contributed to the observed differences in temperature tolerance range between our results and those of others. While the rate of change may result in different thermal limits, our results are supported by field observations and distributions.

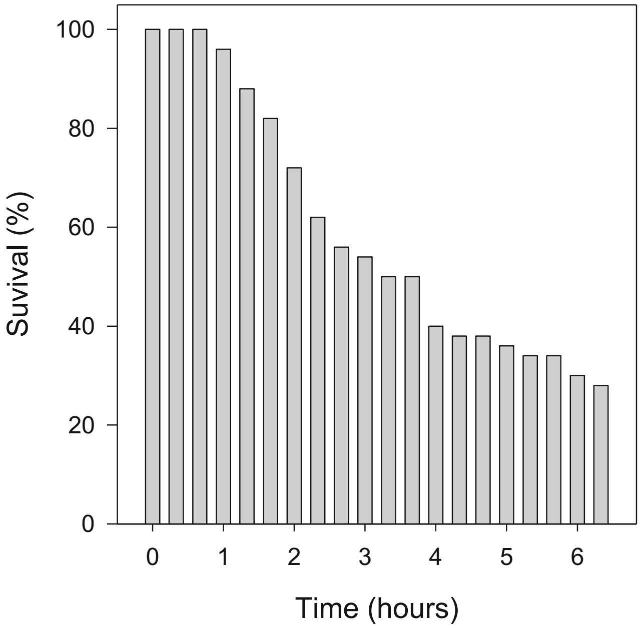

Fig. 6 Cumulative survival of green mussels over time. Survival pooled from both trials over all days with temperature probes and those with no holes drilled

Our salinity tolerance experiments concentrated on lower salinity thresholds; our overall goal was to examine the potential for *P. viridis* to thrive and spread in southwest Florida estuaries. Our results demonstrated that the introduced population of *P. viridis* in southwest Florida can tolerate salinities as low as 6 when allowed to acclimate, but are sensitive to acute drops in salinity of 15 or more. Our experimental design did not include salinities great enough to determine the upper salinity limits of our specimens. However, the reduced survival observed at salinities of 35, compared to that at 30, suggested that salinities of 35 and greater induce physiological stress. Much of the information on salinity tolerance of *P. viridis* is inferred from their distribution limits. In their

Table 1 Surface temperature (°C) and survival (%) of green mussels with no holes or temperature probes over time on three different days

Time (min)	Day 1 (n=12)		Day 2 (n=6)		Day 3 (n=12)	
	Temperature	Survival	Temperature	Survival	Temperature	Survival
0	31	100	35	100	28	100
20	27	100	33	100	33	100
40	29	100	36	100	34	100
60	35	100	40	83.3	33	91.7
80	37	100	40	83.3	35	58.3
100	36	83.3	41	83.3	36	50
120	38	66.7	36	50	37	41.7
140	38	50	36	16.7	37	41.7
160	37	50	40	16.7	35	33.3
180	38	50	40	16.7	34	33.3
200	34	50	38	16.7	37	33.3
220	33	50	38	16.7	38	33.3
240	40	33.3	41	0	33	25
260	39	25				

native range, *P. viridis* occur in estuaries with salinities of 19–44 (Rajagopal et al. 2006; Vakily 1989), and Huang et al. (1983) indicate that salinities of 5 or less limit their distribution in Hong Kong waters. Baker et al. (2006) report established populations in Tampa Bay at salinities as low as 14–16, although they are most abundant in regions of the estuary that average 20–28 (Baker et al. 2012). Results of laboratory studies are mixed and likely reflect varying methods. In acute experiments, Sivalingam (1977) reports near 100 % survival at salinities greater than 24 and salinities less than 80. When salinity is altered gradually, tolerance of *P. viridis* collected in Venezuela is expanded to 0–64 (Segnini de Bravo et al. 1998). However, low salinities may induce physiological stress; at salinities of 12–15 or less, *P. viridis* remain closed, reduce byssal production, halt ammonia excretion, and are unable to reach osmotic equilibrium (McFarland et al. 2013; Rajagopal et al. 2006; this study). Therefore, the results of our laboratory studies, together with previously published work, indicate that the southwest Florida population we sampled has a salinity tolerance range of 12–35.

While the tolerance of *P. viridis* to emersion during cold air temperatures has been reported previously, to our knowledge, ours is the first study to examine the survival of *P. viridis* during aerial exposure at summer temperatures common in southwest Florida. Urian et al. (2011), for example, examined laboratory aerial exposure at temperatures of 21 °C or less and reported significantly increased *P. viridis* mortalities at ≤14 °C. Field observations also suggest that cold air temperatures during emersion are limiting. In Tampa Bay, multiple winter mortality events have been associated with prolonged exposure to air temperatures of less than 2 °C during low water (January 2003—Baker et al. 2012; winters 2007/2008, 2009, and 2010—Firth et al. 2011). Similarly, Power et al. (2004) noted 100 % mortality of intertidal *P. viridis* during winter 2004. Our data show that *P. viridis* were sensitive to high temperatures as well. Even at air temperatures of 25 °C (relatively cool for summer in Florida), our exposed experimental surface frequently exceeded 33 °C and individual *P. viridis* and *C. virginica* both attained internal temperatures as high as 41 °C, at which 100 % mortality was observed in *P. viridis*, but not in *C. virginica*. In contrast, exposed *C. virginica* were hardy and exhibited very low mortality, despite average internal temperatures frequently exceeding 33 °C. Indeed, intertidal oyster reefs in southwest Florida are exposed to prolonged periods of aerial exposure at a wide range of temperatures, ranging from air temperatures of <2 °C to surface temperatures exceeding 50 °C (Goodman et al., unpublished results). Locally, *P. viridis* are only found in the subtidal regions of the bay, while *C. virginica* dominate the intertidal zone. Our results suggest that *P. viridis* are constrained to the deeper portions of the bay where subtidal habitat is available and temperature more stable, leaving the intertidal oyster reefs free from competition.

Implications for *P. viridis* Spread and Competition with *C. virginica*

By assessing the temperature, salinity, and aerial exposure tolerance of *P. viridis*, along with previously published literature, we can make predictions concerning the potential for this invasive bivalve to thrive and spread in southeast US estuaries and to successfully compete with the native oyster. *P. viridis* may not become established any further north than coastal South Carolina, where cold winter temperatures will be limiting (Power et al. 2004; Urian et al. 2011; this study). Our results suggest that *P. viridis* could also encounter lethal and sublethal effects of high temperatures in some estuaries of south Florida, where monthly mean water temperatures in July and August may be as high as 32 °C (NOAA 2014; Surge and Lohmann 2002). Therefore, while climate change may permit range expansion northward (Urian et al. 2011), increasing water temperatures in shallow estuaries coupled with intertidal stress may actually limit the most southern geographic distribution of this species.

While the salinity tolerance of *P. viridis* is relatively broad, low salinities may limit their spread and success in south Florida estuaries. Estuaries in Florida experience dramatic changes in salinity during the summer rainy season, with prolonged periods of salinities below 15 (Barnes et al. 2007). In addition, hurricanes may result in rapid drops in salinity to as low as 0–3 within a few days and continuing for extended periods (Davis et al. 2004; Steward et al. 2006). Acute salinity declines combined with chronic exposure to low salinity, during and following extreme weather events, may temporarily eradicate *P. viridis* from some regions of southwest Florida estuaries. The intolerance of *P. viridis* to acute salinity changes also has implications for their potential transport between estuaries as a fouling organism (Hulme 2009). The success of secondary introductions may be reduced if transport involves a drop in salinity of greater than 15, for example, from one estuary through full marine waters to release in another low-salinity estuary.

Our work contributes to understanding the competitive interactions of *P. viridis* and the eastern oyster, *C. virginica*. *C. virginica* are much more tolerant of extremes in water temperature, salinity, and aerial exposure than are *P. viridis*. *C. virginica* are commonly found in waters with an annual temperature range of −2 to 36 °C (Butler 1985; Galtsoff 1964) and, while *C. virginica* normally occur at salinities of 5–40 (Galtsoff 1964; Wallace 1966), they can survive salinities as low as 2 for up to a month (Gunter 1955). In addition, Galtsoff (1964) and Ingle et al. (1971) report *C. virginica* survival at temperatures as high as 49.5 °C during emersion at low tide. It appears, then, that *P. viridis* competition with *C. virginica* will be limited to subtidal hard substrates in primarily polyhaline regions of estuaries. Intertidal and mesohaline regions of southwest Florida estuaries should represent a refugium from the invasive green mussels, where oyster reefs can persist, free of competition. As a recently introduced species, however, the distribution, population dynamics, and ecosystem impacts of *P. viridis* should continue to be monitored.

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